



European Patent  
Office

SUPPLEMENTARY  
PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

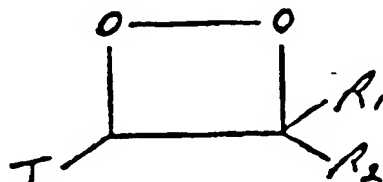
Application Number

EP 89 90 7947

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	TETRAHEDRON LETTERS, vol. 28, no. 44, 1987, pages 5319-5322, Pergamon Journals Ltd, Oxford, GB; R. CURCI et al.: "Synthesis of 1,2-dioxetanes via electron-transfer oxygenation" * The whole article *	1,49	C 07 C 39/14 C 07 C 43/225 C 07 C 43/23 C 07 C 49/788 C 07 D 321/00 C 07 F 7/18 C 07 F 9/12 C 12 Q 1/00 C 12 Q 1/70 G 01 N 21/76 G 01 N 33/542 G 01 N 33/554 G 01 N 33/00
E	WO-A-8 906 226 (QUEST SYSTEMS, INC.) * Claims; pages 5-9,23-25 *	1-49	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 C C 07 D C 07 F
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : 15-18, 22, 23 Claims searched incompletely : 1-14, 19-21, 24-49 Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>The use of undefined wordings in the definition of the radicals in the claims makes a complete search impossible for that type of compounds. For that reason the search has been limited to the examples (see Art. 83 84 EPO) and Rule 45.</p>			
Place of search THE HAGUE		Date of completion of the search 14-11-1991	Examiner HENRY J. C.
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : Intermediate document</p> <p>I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... &amp; : member of the same patent family, corresponding document</p>			

WE CLAIM:

1. A chemiluminescent 1,2-dioxetane compound having the formula:



wherein  $R_1$  is hydrogen, or a bond when  $R_2$  is a substituent bound to the dioxetane ring through a spiro linkage, or an organic substituent that does not interfere with the production of light but satisfies the valence of the dioxetane ring carbon atom to which it is attached;  $R_2$  is a fused polycyclic ring-containing fluorophore moiety bound to the dioxetane ring through a single bond or a spiro linkage and having a labile ring substituent containing a bond which, when cleaved, renders the polycyclic moiety electron-rich to in turn render the dioxetane compound decomposable to emit light, the labile ring substituent's point of attachment to the fused polycyclic moiety, in relation to the fused polycyclic moiety's point of attachment to the dioxetane ring, being such that the total number of ring atoms separating these points of attachment, including the ring atoms at these points of attachment, is an odd whole number; and T is a stabilizing group that prevents the dioxetane compound from decomposing before the labile ring substituent's bond is cleaved.

2. A chemiluminescent 1,2-dioxetane compound as recited in claim 1 in which the odd whole number is 5 or greater.

3. A chemiluminescent 1,2-dioxetane compound as recited in

claim 2 in which the fused polycyclic moiety is the residue of a fused polycyclic aromatic hydrocarbon ring fluorophoric compound containing from 9 to about 30 ring carbon atoms, inclusive.

4. A chemiluminescent 1,2-dioxetane compound as recited in claim 3 in which the residue of a fused polycyclic aromatic hydrocarbon ring fluorophoric compound is a naphthalene residue.

5. A chemiluminescent 1,2-dioxetane compound as recited in claim 3 in which the residue of a fused polycyclic aromatic hydrocarbon ring fluorophoric compound is an anthracene residue.

6. A chemiluminescent 1,2-dioxetane compound as recited in claim 2 in which the fused polycyclic moiety is the residue of a less than fully aromatic fused polycyclic hydrocarbon ring fluorophoric compound containing from 10 to about 30 carbon atoms, inclusive.

7. A chemiluminescent 1,2-dioxetane compound as recited in claim 6 in which the residue of a less than fully aromatic fused polycyclic hydrocarbon ring fluorophoric compound is a fluorene residue.

8. A chemiluminescent 1,2-dioxetane compound as recited in claim 2 in which the fused polycyclic moiety is the residue of a fused polycyclic heterocyclic ring fluorophoric compound containing from 9 to about 30 ring atoms, inclusive.

9. A chemiluminescent 1,2-dioxetane compound as recited in claim 8 in which the residue of a fused polycyclic heterocyclic ring fluorophoric compound is a quinoline residue.

10. A chemiluminescent 1,2-dioxetane compound as recited in any one of claims 3-9, inclusive, in which  $R_1$  is a methoxy group,  $R_2$  is bound to the dioxetane ring through a single bond

and T is an adamant-2-ylidene group.

11. A chemiluminescent 1,2-dioxetane compound as recited in claim 10 in which the labile ring substituent is enzymatically cleavable.

12. A chemiluminescent 1,2-dioxetane compound as recited in claim 11 in which the labile ring substituent is a phosphate ester group.

13. A chemiluminescent 1,2-dioxetane compound as recited in claim 11 in which the labile ring substituent is a galactoside group.

14. A chemiluminescent 1,2-dioxetane compound as recited in claim 10 in which the labile ring substituent is chemically cleavable.

15. A 3-(2'-spiroadamantane)-4-methoxy-4-(6"-phosphoryl-oxy)naphth-1'-yl-1,2-dioxetane salt.

16. A 3-(2'-spiroadamantane)-4-methoxy-4-(7"-phosphoryl-oxy)naphth-2'-yl-1,2-dioxetane salt.

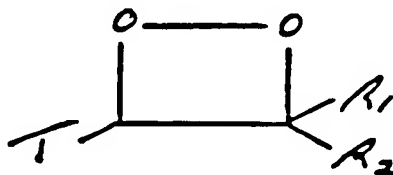
17. A 3-(2'-spiroadamantane)-4-methoxy-4-(8"-phosphoryloxy)naphth-1'-yl-1,2-dioxetane salt.

18. A 3-(2'-spiroadamantane)-4-methoxy-4-(5"-phosphoryloxy)naphth-2'-yl-1,2-dioxetane salt.

19. The disodium salt of a 1,2-dioxetane compound as recited in claim 14.

20. A method for generating light which comprises:

(a) providing a chemiluminescent 1,2-dioxetane compound having the formula:



wherein  $R_1$  is hydrogen, or a bond when  $R_2$  is a substituent bound to the dioxetane ring through a spiro linkage, or an organic substituent that does not interfere with the production of light but satisfies the valence of the dioxetane ring carbon atom to which it is attached;  $R_2$  is a fused polycyclic ring-containing fluorophore moiety having a labile ring substituent containing a bond which, when cleaved, renders the fused polycyclic moiety electron-rich to in turn render the dioxetane compound decomposable to emit light, the labile ring substituent's point of attachment to the fused polycyclic moiety, in relation to the fused polycyclic moiety's point of attachment to the dioxetane ring, being such that the total number of ring atoms separating these points of attachment, including the ring atoms at these points of attachment, is an odd whole number; and T is a stabilizing group that prevents the dioxetane compound from decomposing before the labile ring substituent's bond is cleaved, and

(b) decomposing the 1,2-dioxetane compound by cleaving the labile ring substituent's bond.

21. A method for generating light as recited in claim 20 in which the labile ring substituent's bond is cleaved by an enzyme.

22. A method for generating light as recited in claim 21 in which the 1,2-dioxetane compound is a 3-(2'-spiroadamantane)-4-methoxy-4-(6"-phosphoryloxy)naphth-1'-yl-1,2-dioxetane salt, a 3-(2'-spiroadamantane)-4-methoxy-4-(7"-phosphoryloxy)naphth-2'-yl-1,2-dioxetane salt, a 3-(2'-spiroadamantane)-4-methoxy-4-(8"-phosphoryloxy)naphth-1'-yl-1,2-dioxetane salt or a 3-(2'-spiroadamantane)-4-methoxy-4-(5"-phosphoryloxy)naphth-2'-yl-1,2-dioxetane salt.

23. A method for generating light as recited in claim 22 in which the 1,2-dioxetane salt is a disodium salt.

24. In a process in which light released by the decomposition of a chemiluminescent chemical compound is detected to determine the presence, concentration or structure of an analyte, the improvement comprising carrying out the process in the presence of at least one chemiluminescent 1,2-dioxetane compound as recited in claim 1.

25. The process of claim 24 in which the process carried out is a step in an immunoassay.

26. The process of claim 25 in which the immunoassay is for the detection of a specific binding pair comprising an antigen and an antibody.

27. The process of claim 25 in which the label used in the assay is an enzyme.

28. The process of claim 25 in which the label used in the assay is the chemiluminescent 1,2-dioxetane compound.

29. The process of claim 25 in which the chemiluminescent 1,2-dioxetane compound is enzymatically cleavable.

30. The process of claim 25 in which the chemiluminescent 1,2-dioxetane compound is chemically cleavable.

31. The process of claim 25 in which the immunoassay is for the detection of an enzyme.

32. The process of claim 25 in which the immunoassay is for the detection of a hormone.

33. The process of claim 24 in which the process carried out is a step in a chemical assay.

34. The process of claim 33 in which the chemical assay is for the detection of a chemical substance which, during the assay, is caused to decompose to form a substance capable of causing the chemiluminescent 1,2-dioxetane compound to decompose.

35. The process of claim 34 in which the chemical substance is glucose.

36. The process of claim 34 in which the chemical substance is cholesterol.

37. The process of claim 24 in which the process carried out is a nucleic acid probe assay.

38. The process of claim 37 in which the nucleic acid probe assay is for the detection of a virus.

39. The process of claim 24 in which the process carried out is a histocompatibility assay.

40. The process of claim 24 in which the process carried out is a technique for studying the microstructure of a macromolecule.

41. The process of claim 24 in which the process carried out is a multi-channel assay carried out in the presence of at least two of the chemiluminescent 1,2-dioxetane compounds as recited in claim 1 as substrates, each of which upon decomposition emits light of a different wavelength from the other(s) and each of which has a labile ring substituent cleavable by a different means from the other(s).

42. The process of claim 24 in which the process carried out is a multi-channel assay carried out in the presence of at least one of the chemiluminescent 1,2-dioxetane compounds as recited in claim 1 and at least one other chemiluminescent

compound as substrates, each of which chemiluminescent 1,2-dioxetane compounds and other chemiluminescent compounds emit light of a different wavelength from the other(s) and each of which is cleavable by different means from the other(s) to produce a light-emitting fluorophore moiety.

43. The process of claim 42 in which the multi-channel assay is an immunoassay for the quantitation of human chorionic gonadotropin and human luteinizing hormone contained in a single sample and is carried out in the presence of 3-(2'-spiroadamantane)-4-methoxy-4-(3'-phosphoryloxy)phenyl-1,2-dioxetane disodium salt and 3-(2'-spiroadamantane)-4-methoxy-4-(7"-acetoxy)naphth-2'-yl-1,2-dioxetane as the chemiluminescent substrates.

44. The process of claim 43 in which the phosphoryloxyphenyl dioxetane chemiluminescent substrate is cleaved by alkaline phosphatase conjugated to mouse monoclonal anti- $\beta$ -HCG and the acetoxynaphthyl dioxetane chemiluminescent substrate is cleaved by carboxylesterase conjugated to mouse monoclonal anti-HLH.

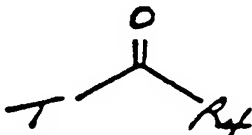
45. The process of claim 42 in which the multi-channel assay is a nucleic acid probe assay for the quantitation of herpes simplex virus, cytomegalovirus and human papiloma virus contained in a single sample and is carried out in the presence of 3-(2'-spiroadamantane)-4-methoxy-4-(3'-phosphoryloxy)phenyl-1,2-dioxetane disodium salt, 3-(2'-spiroadamantane)-4-methoxy-4-(7"-acetoxy)naphth-2'-yl-1,2-dioxetane and 3-(2'-spiroadamantane)-4-methoxy-4-(5"- $\beta$ -galactosyloxy)naphth-2'-yl-1,2-dioxetane as the chemiluminescent substrates.



46. The process of claim 45 in which the phosphoryloxyphenyl dioxetane chemiluminescent substrate is cleaved by a DNA probe for HSV labeled with alkaline phosphatase, the acetoxynaphthyl dioxetane chemiluminescent substrate is cleaved by a DNA probe for CMV labeled with carboxylesterase and the  $\beta$ -galactosyloxynaphthyl dioxetane chemiluminescent substrate is cleaved by a DNA probe for HPV labeled with  $\beta$ -galactosidase.

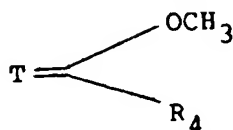
47. A fused polycyclic hydrocarbon ring-containing fluorophoric compound having from 9 to 30 carbon atoms, inclusive, and having a halo substituent and an ether substituent or a hydroxy substituent, the total number of ring carbon atoms separating said two substituents, including the carbon atoms to which said substituents are attached, being an odd whole number.

48. A compound having the general formula:



wherein T is an unsubstituted or substituted cycloalkyl, aryl, heteroaryl, alkoxy, aryloxy, alkoxyalkoxy, cycloalkylidene or fused polycycloalkylidene group and R<sub>4</sub> is the residue of a fused polycyclic hydrocarbon ring-containing fluorophoric compound having from 9 to about 30 carbon atoms, inclusive, and having an ether substituent, the total number of carbon atoms separating the ring carbon atom to which said ether substituent is attached and the ring carbon atom through which R<sub>4</sub> is attached to the remainder of the compound, including the carbon atoms at said points of attachment, being an odd whole number.

49. A compound having the general formula:



wherein T is a substituted or unsubstituted cycloalkylidene or fused polycycloalkylidene group and R<sub>1</sub> is the residue of a fused polycyclic hydrocarbon ring-containing, fluorophore-forming group having from 9 to about 30 carbon atoms, inclusive, and having an ether substituent, the total number of carbon atoms separating the ring carbon atom to which said ether substituent is attached and the ring carbon atom through which R<sub>4</sub> is attached to the remainder of the compound, including the carbon atoms at said points of attachment, being an odd whole number.